The Morphology and Chemical Composition of Horny Fiber from an Unidentified Creature Captured off the Coast of New Zealand*

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Abstract: The only material evidence, horny fiber, of an unidentified creature captured off the coast of New Zealand was characterized with respects to its hydrothermal behaviour, electron microscope observation, and chemical composition. From the combined results, the horny fiber was identified as a unique collagenous fiber, elastoidin, which has been reported to occur only in shark fins. Especially, it was almost identical to a basking shark elastoidin in its amino acid composition. Thus, these findings may lead us to the conclusion that the unidentified creature is a basking shark or a closely related species to it.

On April 25, 1977, the Zuiyo Maru of Taiyo Fishery Company, a Japanese fishing trawler, happened to drag up the corpse of a big, strange creature resembling the legendary "Nessie" about 30 miles off the coast of Christchurch, New Zealand. The creature measured up to 10 meters long. The dead body, however, was soon thrown away for fear of contaminating other fish with putrid substances. Just before it was thrown away, Michihiko Yano, one of the crewmen, had pulled out several horny fibers from the half-decayed pectoral fin (or foreleg). These horny fibers resemble a unique collagenous fiber, elastoidin, from the fins of shark (Class Chondrichthyes) in their appearance. On the other hand, Yano's color-photographs and sketches of the creature as well as many verbal reports of the crewmen seem to support the possibility that it is not a fish. Thus, it would be expected that a biochemical study on the isolated horny fiber may provide significant information about this unidentified creature. The present paper reports the morphological and chemical characteristics of the horny fiber in relation to those of shark elastoidin.

Materials and Methods

Samples. Two pieces of the horny fibers from the pectoral fin (about 100 cm in length) of the creature were kindly provided by Professor Fujio Yasuda, Tokyo University of Fisheries, who was requested by Taiyo Fishery Company to give an opinion on the creature. According to Yano's report, the horny fibers were washed with water in order to remove their terrible smell, treated with an antiseptic solution of about 0.04% sodium hypochlorite (NaClO) at room temperature for 12 h, and then air-dried on the trawler.

Direct comparison was made with elastoidin from the basking shark *Cetorhimus*

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maximus, because some adult basking sharks are comparable to the creature in their whole-body length. Elastoidins were prepared from the fresh pectoral fin (about 40 cm in length) and any loose substance surrounding the elastoidin was removed by washing with water.

Electron microscopy. The horny fibers were macerated in 1% acetic acid, washed with physiological saline, and fixed for 2 h with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at 4°C. After washing with the same buffer, they were postfixed for 1 h in 1% osmium tetraoxide at 4°C. A portion of them was ion-spattered with gold and examined in a U–3 Type scanning electron microscope (JEOL Co. Ltd.). Another portion was dehydrated in an ethanol series, embedded in spurr resin, and sectioned longitudinally. These sections were stained with lead citrate and uranyl acetate, and examined in a HS–9 transmission electron microscope (HITACHI Co. Ltd.).

Chemical analysis. Amino acid compositions of the horny fiber and the shark elastoidin were determined by an amino acid analyzer, JLC-3BC liquid chromatograph (JEOL Co. Ltd.). The samples were hydrolyzed in 6 N HCl at 110°C for 24 h under reduced pressure. Two kinds of carbohydrate-linked hydroxylysines, glucosylgalactosylhydroxylysine (Glc-Gal-Hyl) and galactosylhydroxylysine (Gal-Hyl), were estimated by the amino acid analyzer according to the method as described by Kimura (1972) in samples hydrolyzed in 2 N KOH at 105°C for 20 h. Furthermore, reducible cross-links were identified using the method reported by Tanzer *et al.* (1973); the samples were reduced with tritiated sodium borohydride (NaB^3H_4), hydrolyzed in 3 N HCl at 107°C for 48 h, and then fractionated on a 0.9 × 15 cm column of Aminex A-5.

Results and Discussion

Morphology. The horny fibers are rigid, needle-like fibers with a translucent light-brown color, which taper towards both ends (Fig. 1). This fiber shrinked sharply to about 1/3 of the original length at approximately 63°C, when heated in water. On cooling, the shrunk fiber became re-elongated gradually. Fig. 2 compares the wet horny fibers before

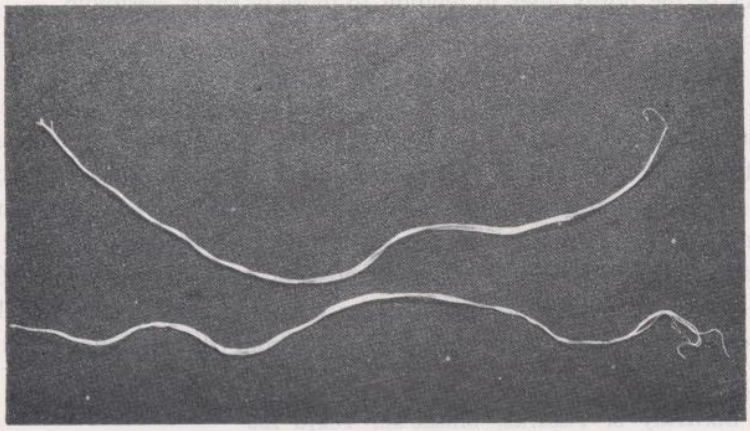

Fig. 1. Air-dried horny fibers isolated from the unidentified creature captured off the coast of New Zealand.

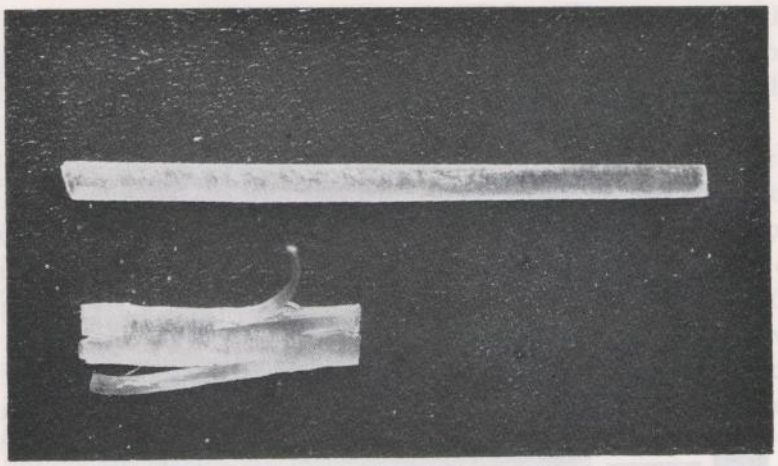

Fig. 2. Thermal shrinkage of the horny fiber in hot water. Upper: native horny fiber; Lower: shrunk horny fiber treated at 80°C for 15 sec.

and after heat treatment, both having the same original length of 3.5 cm. This type of hydrothermal behaviour was characteristic of elastoidin (Faure-Fremiet, 1937; Damodaran *et al.*, 1956). In this connection, the basking shark elastoidin was found to have a shrinkage temperature of approximately 65°C.

The scanning electron micrograph shown in Fig. 3 exhibited that the horny fiber was composed of numerous protofibrils arrayed parallel to the fiber axis. Furthermore, the transmission electron micrograph of longitudinal section of the horny fiber is given in Fig. 4, in

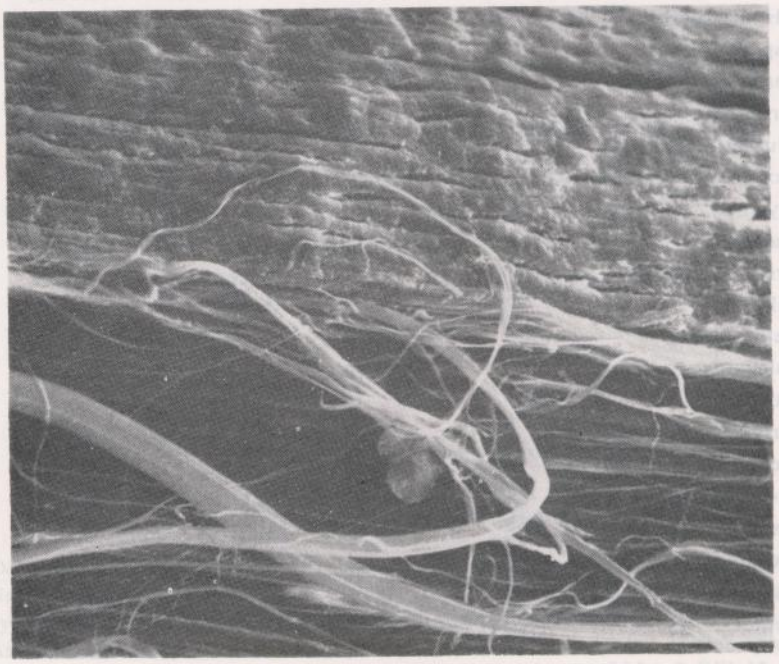

Fig. 3. Scanning electron micrograph of the horny fiber, ×300.

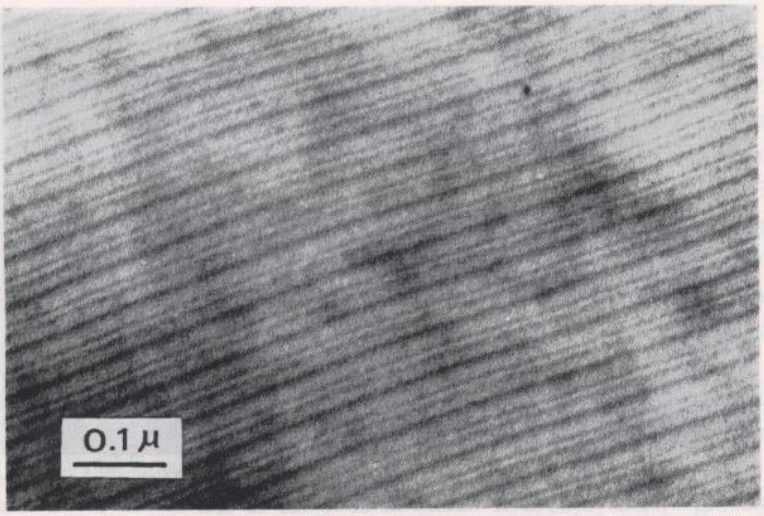

Fig. 4. Transmission electron micrograph of longitudinal section of the horny fiber, ×140,000. The section was stained with lead citrate and uranyl acetate. The fiber axis is perpendicular to the banding.

Table 1. Amino acid composition of the horny fiber and the basking shark elastoidin

	Horny fiber	Elastoidin	
		Native	NaClO treatment*
	(residues/1000 residues)		
4-Hydroxyproline	45	45	45
Aspartic acid	54	56	55
Threonine	25	25	25
Serine	39	40	40
Glutamic acid	80	80	80
Proline	130	125	125
Glycine	291	282	290
Alanine	109	106	110
Cystine (1/2)	7	8	6
Valine	25	24	24
Methionine	10	10	10
Isoleucine	20	20	20
Leucine	19	19	19
Tyrosine	43	48	41
Phenylalanine	12	12	12
Hydroxylysine	5	6	6
Lysine	25	26	26
Histidine	11	15	13
Arginine	51	53	53
(Amide-N)	(57)	(80)	(62)

* 0.007% NaClO for 24 h at room temperature.

which the peculiar band-pattern was observed over the entire field of view in the electron microscope. The major periodic striation of 450–500 Å was rather shorter than that of 600–700 Å for typical collagens. A similar characteristic pattern was previously observed by McGavin (1962) in the longitudinal section of basking shark elastoidin.

Chemical characteristics. The amino acid composition of the horny fiber is shown in Table 1, indicating that more than 95% of the horny fiber by weight was accounted for these amino acids. Although its composition was characteristic of collagenous proteins, an unusual feature of the horny fiber was found to be the high tyrosine content of 43 residues/1000 amino acid residues, comparing with about 5 residues or less/1000 residues for collagens. In this respect, the horny fiber resembled elastoidins thus far known (Damodaran *et al.*, 1956; Kimura and Kubota, 1966). Especially, the basking shark elastoidin listed in Table 1 was closely similar to the horny fiber, although it was obviously rich in tyrosine and histidine as compared with the horny fiber.

In this regard, it is noteworthy that the horny fiber preparation had been treated with a dilute NaClO solution which is a strong oxidising reagent. The aqueous NaClO solution is very unstable, so that the NaClO concentration, 0.04%, reported by Yano should be considered as maximal. The action of NaClO on collagenous proteins is not yet clear, but some amino acids were reported to be susceptible to the oxidation by chemical oxidising reagents (Ignac and Joseph, 1967). Thus, the basking shark elastoidins were treated with 0.04% and 0.007% solutions of NaClO at room temperature for 24 h and then analyzed for their amino acids. Fig. 5 shows that tyrosine, histidine, cystine, and methionine residues of the elastoidin were specifically decomposed by the action of NaClO, whereas other amino acid residues remained intact. Thus, the horny fiber was found

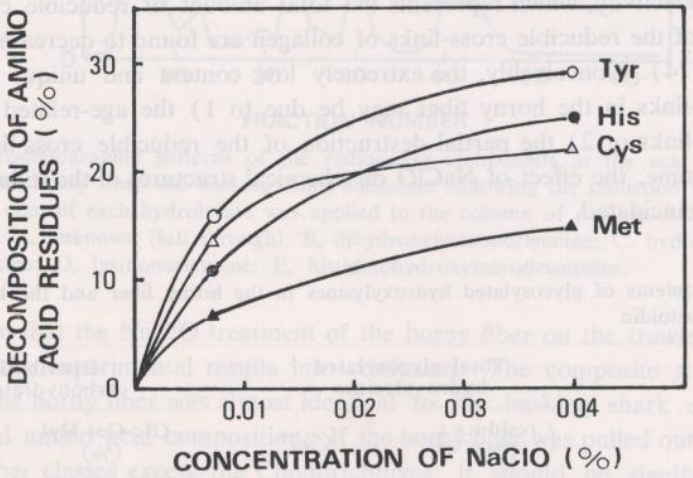

Fig. 5. Decomposition of amino acid residues in the basking shark elastoidin treated with NaClO. Native elastoidins were treated with NaClO at room temperature for 24 h, hydrolyzed in 6 N HCl at 110°C for 24 h, and then analyzed for their amino acids. Amino acid residues other than tyrosine (Tyr), histidine (His), cystine (Cys), and methionine (Met) remained intact.

to have a strong resemblance to the elastoidin treated with 0.007% NaClO rather than the intact elastoidin in its amino acid composition (Table 1). This striking similarity was confirmed by "a difference index (DI)" which was proposed by Metzger *et al.* (1968). That is, the DI calculated for the horny fiber and the NaClO-treated elastoidin was the extremely low value of 0.95, suggesting that both proteins were almost identical with each other.

The results of the determinations of the carbohydrate-linked hydroxylysines in the horny fiber and the elastoidin are presented in Table 2. No significant difference was observed; they contained the total glycosylated hydroxylysine of approximately 2 residues/1000 residues, and about 40% of their total hydroxylysine was glycosylated. Moreover, the distribution of the disaccharide unit, Glc-Gal, and the monosaccharide unit, Gal, was found to be about 1 : 1 in both samples.

In contrast to the amino acid composition described above, there was a marked difference between the horny fiber and the elastoidin in their reducible cross-links which are polyfunctional amino acids derived from lysine, hydroxylysine, and/or histidine residues. After the reduction with NaB^3H_4, the radioactivity of tritium incorporated into the horny fiber was about 110 cpm per μg of hydroxyproline, which was 1/7 of the specific radioactivity of the elastoidin. This fact indicated that the horny fiber contained the extremely low amount of reducible cross-links, comparing to the elastoidin. Chromatographic patterns of the radioactive-compounds (Fig. 6) showed that each of the horny fiber and the elastoidin exhibited a unique pattern of the reducible cross-link formation. The horny fiber contained the comparable amounts of hydroxylysinonorleucine, lysinonorleucine, and histidinohydroxymerodesmosine, which are commonly observed in skin and tendon collagens. On the other hand, lysinonorleucine was found to be the major reducible cross-link in the elastoidin. It is generally said that reducible cross-links are liable to decompose under relatively mild conditions (Bailey *et al.*, 1970). In addition, it has been reported that the specific radioactivity, which represents the total amount of reducible compounds, and the proportion of the reducible cross-links of collagen are found to decrease with age (Fujii and Tanzer, 1974). Conceivably, the extremely low content and unique composition of reducible cross-links in the horny fiber may be due to 1) the age-related changes in the reducible cross-links or 2) the partial destruction of the reducible cross-links by NaClO. At the present time, the effect of NaClO on chemical structures of the reducible cross-links remains to be elucidated.

Table 2. Contents of glycosylated hydroxylysines in the horny fiber and the basking shark elastoidin

	Total glycosylated hydroxylysines		Distribution of carbohydrate units*1	
	$\left(\frac{\text{residues}}{1000}\right)$	(%)*2	Glc-Gal-Hyl (%)	Gal-Hyl (%)
Horny fiber	1.9	41	49	51
Elastoidin	2.2	39	49	51

*1 Calculated from total glycosylated hydroxylysines and total hydroxylysines.
*2 Expressed as molar percentage.

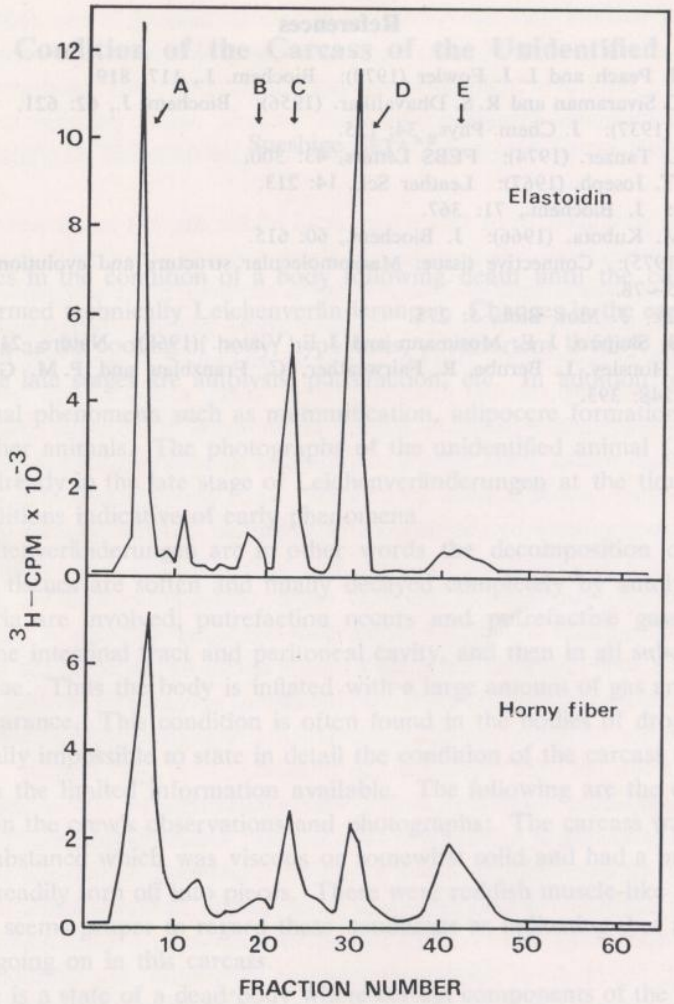

Fig. 6. Chromatographic patterns of the radioactive compounds in the acid hydrolysates of the horny fiber and basking shark elastoidin following the reduction with NaB^3H_4. 10^5 cpm of each hydrolysate was applied to the column of Aminex A-5. The peaks are: A, unknown (fall through); B, dihydroxylysinonorleucine; C, hydroxylysinonorleucine; D, lysinonorleucine; E, histidinohydroxymerodesmosine.

It is certain that the NaClO treatment of the horny fiber on the trawler threw our interpretation of the experimental results into confusion. The composite studies, however, indicated that the horny fiber was almost identical to the basking shark elastoidin in its morphology and amino acid composition. If the horny fiber was pulled out from an animal belonging to other classes except the Chondrichthyes, it should be significantly different from the basking shark elastoidin in its amino acid composition, judging from the present knowledge of comparative biochemistry of collagenous protein (Mathews, 1975). These results strongly suggest that this unidentified creature is a basking shark or a closely related species to it.

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